Molecular Pathogenesis of Myelodysplastic Syndromes

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Abstract - **Myelodysplastic syndromes (MDS) are a group of clonal hematologic disorders characterized by inefficient hematopoiesis, hypercellular bone marrow, dysplasia of blood cells and cytopenias. Most patients are diagnosed in their late 60s to early 70s. MDS is a risk factor for the development of acute myeloid leukemia which can occur in 10-15% of patients with MDS. A variety of pathophysiologic mechanisms contributes to the genesis and persistence of MDS including immunologic, epigenetic, cytogenetic and genetic factors. The only potential curative option for MDS is hematopoietic cell transplantation which is suitable for only a few patients. Currently approved therapeutic options for MDS, including lenalidomide, decitabine, and 5-azacytidine, are targeted to improve transfusion requirements and quality of life. Moreover, 5-azacytidine has also been demonstrated to improve survival in some patients with higher risk MDS. New ways to predict which patients will better gain benefit from currently available therapeutic agents are the primary challenges in MDS. In the last 10 years, chromosome scanning and high throughput technologies (single nucleotide polymorphism array genotyping, comparative genomic hybridization, and whole genome/ exome sequencing) have tremendously increased our knowledge of MDS pathogenesis. Indeed, the molecular heterogeneity of MDS supports the idea of different therapeutic approaches which will take into account the diverse morphologic and clinical presentations of MDS patients rather than a restricted therapeutic strategy. This review will summarize the molecular abnormalities in key relevant components of the biology and pathogenesis of MDS and will provide an update on the clinical impact and therapeutic response in MDS patients.**

Keywords: MDS, pathogenesis, molecular mutations

I. INTRODUCTION OVERVIEW OF MYELODYSPLASTIC SYNDROMES

Myelodysplastic syndromes (MDS) are a group of clonal myeloid disorders which are morphologically characterized by bone marrow (BM) hypercellularity, uni- or multilineage dysplasia, and peripheral blood cytopenias. The incidence rate of MDS in the United States for the years 2003-2007 has been estimated at 4.3 per 100, 000 people which accounts for 15,000 new cases every year [1,2]. Statistics on incidence rates might be higher due to misdiagnosis of cases with hypocellular BM or misinterpretation of the anemia observed in MDS as a normal condition in the elderly. It is a disease more frequently diagnosed in men with the exception of MDS with 5q- syndrome, which is slightly more frequent in women. Chromosomal defects are usually clonal and recurrent. They are observed in approximately 50% of primary and 80% of cases of therapy-related MDS (t-MDS) [3,4]. The most commonly affected chromosomes are 5, 6, 7, 8, 11, 13 and 20 and the most recurrent chromosomal lesions are partial deletions (5, 7, 11, 13, and 20) and additional copies (6 and 8). Unbalanced translocations are also sometimes seen. Single chromosomal alterations are usually detected in *de novo* MDS while a complex karyotype, defined as having $\geq$3 chromosomal abnormalities is usually observed in MDS patients with antecedent exposure to treatments such as chemo/ radiotherapy or to toxic chemicals. The latter condition is included in the MDS category called secondary MDS (sMDS) which includes MDS that evolved from a prior hematologic disorder. The impact of having multiple chromosomal anomalies has also been investigated and correlated with unfavorable outcome in patients with sMDS. Cytogenetics has a crucial impact on the outcome of patients with MDS and acute myeloid leukemia (AML) undergoing hematopoietic stem cell transplantation [5]. The accumulation of genetic defects in addition to the primary molecular mutation affecting a stem cell increases the propensity to develop more aggressive diseases like AML. The importance of cytogenetic abnormalities in MDS is also due to the fact that chromosomal regions often contain genes relevant to MDS biology and pathophysiology. Indeed, the presence of chromosomal abnormalities like deletions suggests that haploinsufficiency and/ or loss of tumor suppressor genes (TSGs) are mechanisms important in MDS biology.

Conventional Sanger sequencing, high resolution whole genome scanning technologies (array-based comparative genomic hybridization (aCGH) and single nucleotide polymorphism arrays (SNP-A) genotyping) and high-throughput sequencing technologies (whole genome/ exome sequencing) have brought to light molecular alterations in genes of numerous pathways including methylation, transcriptional factors, signal transduction, histone regulators, and the RNA splicing machinery [6]. The diversity of these lesions and their combinations may reflect the heterogeneity in the morphologic presentations, clinical courses, and potentially response to therapeutic agents. Ultimately, the finding of genes relevant to MDS

pathophysiology also opens the possibility of targeted therapy.

II. LOW AND HIGH RESOLUTION TECHNOLOGIES

The World Health Organization (WHO) has classified MDS into several distinct disease groups differentiated from others by BM and peripheral blood (PB) morphology (blasts, ring sideroblasts, dysplasia, cytopenias) and cytogenetic changes. Different risk stratification scoring systems have been created and revised for *de novo* MDS. Metaphase cytogenetics (MC) still serves as an important weighted parameter in the risk stratification assessment (Figure 1). Classically, MC is the gold standard in the determination of cytogenetic abnormalities in myeloid cancers. The advantages of MC include the simplicity of the method, the detection of unbalanced/ balanced chromosomal defects, and the feasibility in discriminating single cellular clones. However, MC reaches a sensitivity of only 10% and it is informative in only 46-59% of patients with MDS due to the limitation in achieving results when cells are unable to grow or in case of non-informative karyotypes [7.8]. Further, even if one-half of MDS patients have a normal karyotype by MC, this does not necessarily exclude the presence of latent genetic defects not clinically dominant which escape detection due to the low resolution of MC. A combination of chromosome painting and multi-color fluorescence called spectral karyotyping (SKY) has complemented the photographic images of condensed chromosomes by G-banding and fluorescence *in situ* hybridization (FISH) for specific chromosome (5, 7, and 20) and improved the detection of chromosomal rearrangements and minimal monosomies leading to a better picture of the karyotype [9].

Advances in high resolution genome scanning technologies like aCGH and SNP-A have improved the detection rate of genomic lesions in MDS and other related disorders and have clarified many aspects of MDS biology [10]. aCGH is a technology that identifies differences in copy number between DNA of patients and

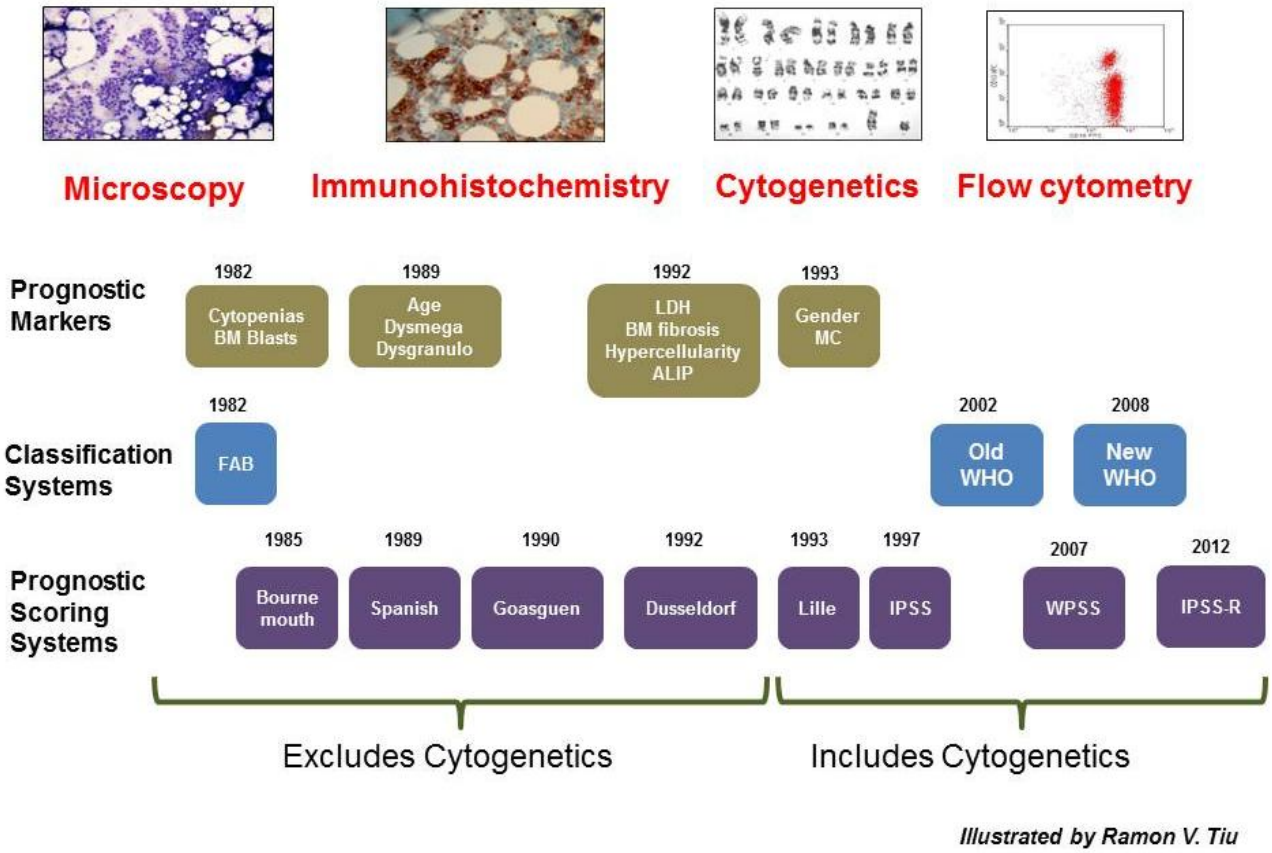

Figure 1. Schema of the diagnostic tests, classification systems, and prognostic/ scoring systems in Myelodysplastic syndromes. Microscopy, immunohistochemistry, G-banding, and sometimes flow cytometry are all useful diagnostic tests in Myelodysplastic syndromes (MDS). Prognostic markers include: bone marrow and peripheral blood morphology, numbers of blasts, morphologic features (ring sideroblasts), dysplasia, cytopenias and cytogenetic changes. Classification systems include (FAB, 2002 and 2008 WHO). Risk stratification scoring systems have been created for *de novo* MDS with and without account for cytogenetic findings.

Abbreviations. BM, bone marrow; LDH, lactate dehydrogenase; ALIP, abnormal localization of immature precursors; MC, metaphase cytogenetics; FAB, French American British Classification; IPSS, International prognostic scoring system; WPSS, WHO classification-based Prognostic Scoring System; IPSS-R: International prognostic scoring system-Revised.

healthy subjects on the basis of fluorescent hybridization signals. SNP-A has combined two techniques commonly used in the DNA microarray technologies, DNA hybridization and fluorescence. The use of SNP-A has overcome a limitation of the aCGH technology and provides the ability to detect a cryptic cytogenetic defect called acquired somatic uniparental disomy (AS-UPD) which is recurrent in 20% of patients with MDS. Several studies have reported the clinical importance of both aCGH and SNP-A on the survival settings of MDS [11-14]. However, efforts have been made towards the combination of all these approaches with MC and several multicenter studies have highlighted the worse survival outcomes of patients with MDS and other disorders carrying defects detected by MC/ SNP-A in addition to several other studies where the number of defects by SNP-A predict worse outcomes [15-18].

More recently, high-throughput sequencing, such as whole genome/ exome and deep sequencing, has been instrumental in discovering germ-line and somatic variants in a number of solid tumors and blood cancers including MDS. These technologies are able to generate a comprehensive sequence database of an individual (whole genome), a targeted genome re-sequencing which allows for the generation of specific sequences (whole exome) or a sequence of the entire genome with higher ''depth'' indicating that the process of the sequencing is performed more than one time for any one region of the genome (deep sequencing). This latest approach has markedly improved the accuracy of sequencing, reducing the error output.

III. BIOLOGICAL AND CLINICAL IMPACT OF MOLECULAR MARKERS IN MDS

More than a decade ago, cytogenetic defects were the foremost determinant of MDS biology. The advancement in high resolution genomic technologies helped in the identification of regions of the genome highly suspected to contain molecular mutations. This was the case for the original discoveries of *TET2*, *EZH2*, *CBL*, *IDH1*/ *IDH2*, *ASXL1*, *DNMT3A*, *UTX*, *TP53*, *SF3B1*, *U2AF1*, and *SETBP1* (Table 1).

We will discuss the role of several of these genes and pathways for which the biological and clinical impact has been studied and associated with MDS pathophysiology.

IV. COMMONLY AFFECTED PATHWAYS: METHYLATION PATHWAY: TET2, DNMT3A, IDH1/2

Aberrant DNA methylation has been observed in MDS. Methylation of promoters at CpG loci is one of the mechanisms that can regulate and silence TSGs. The identification of distinct methylation patterns, hypermethylation of key genes important in differentiation, and the success of using DNA-methyltransferase inhibitors (5-aza-2'-deoxycytidine and 5-azacytidine) in some MDS patients provides the rationale supporting the key role of methylation as a cru cial regulatory mechanism in MDS. Indeed, several studies have demonstrated that the increase in aberrant DNA methylation and chromosomal anomalies is directly proportional to the malignant transition of MDS to AML [19,20].

TABLE 1. FREQUENCY OF MOLECULAR MUTATIONS IN MYELOID MALIGNANCIES

Gene Name	Frequency MDS %	Frequency MDS/ MPN %	Frequency AML %
TET2*	12 – 14	37 – 46	9 – 43
IDH1/2*	1 – 5	9	5 – 10
DNMT3A*	8	12	25
ASXL1*	21	17 – 46	17
EZH2	2	4 – 12	1
CBL	1 - 2	5	9
SF3B1*	4[§]	7[#]	5 – 6
U2AF1*	6 – 12	8 – 17	1 – 10
SRSF2*	6 – 12	28 – 47	1 – 7
TP53*	8	7	56 – 78
NRAS/KRAS	4/1	7/4	9 – 40/ 5 - 17
RUNX1*	9	13	13
SETBP1*	4.1	9.4	0.9-9.1[†]

*Indicates the genes discussed in the text. [§]68% in refractory anemia with ring sideroblasts. [#] 81% on refractory anemia with ring sideroblasts associated with thrombocytosis. [†]frequency for *de novo* and secondary AML, respectively.

Abbreviations. MDS, Myelodysplastic syndromes; MDS/ MPN, Myelodysplastic syndromes/ Myeloproliferative neoplasms; AML, Acute myeloid leukemia.

Genes that act as regulators of the DNA methylation have been found to be frequently mutated in MDS. SNP-A identified a region of loss of heterozygosity on chromosome 4q24 which contains a gene called *TET2* which is frequently mutated in MDS. *TET2* was mutated in 20-25% of MDS patients [21,22]. *TET2* is a dioxygenase that catalyzes the conversion of the modified base 5-methylcytosine (5-MC) to 5-hydroxymethylcytosine (5-hMC) by oxidizing 5-MC. The conversion of 5-MC to 5hMC represents the first step in cytosine demethylation. Indeed, methylation at the C5 position of cytosine appears to be an epigenetic modification which plays an important role in transcriptional regulation. *TET2* mutations are restricted to the C- terminus of the protein, resulting in loss of function which correlates with a decrease of 5-hMC levels and an increase of 5-MC leading to DNA hypermethylation and gene silencing [23,24]. Even though *TET2* mutations are correlated with low 5-hMC levels in myeloid disorders, a fraction of patients with wild-type *TET2* also have low 5-hMC levels. A recent report correlated a low level of 5-hMC to the over-expression of the ancestral CXXC domain of *TET2* which encodes for a specific gene called *IDAX* [25]. In addition, several reports have described that

low 5-hmC levels are associated with DNA hypomethylation in patients with myeloid malignancies while others have described DNA hypermethylation in AML [23]. A mouse model of *TET2* loss of function shows increased proliferation of myeloid cells without manifesting any dysplastic changes resembling MDS [26]. The clinical impact of *TET2* mutations has been widely studied by many groups and somehow is still under debate. (Table 2) *TET2* mutations do not seem to predict an increased risk for transformation to AML. Most studies looking at the prognostic value of *TET2* in MDS did not show a difference in survival outcomes between mutant and wild type cases. The efficacy and effectiveness of hypomethylating therapy in specific subsets of MDS patients and the presence of molecular mutations in genes relevant to methylation also justifies the efforts in genotyping large cohorts of patients with MDS receiving hypomethylating drugs for these specific molecular changes. Itzykson and colleagues showed that high-risk MDS patients carrying *TET2* mutations have better response rate to 5-azacytidine without any improvement in OS [27]. We demonstrated in a cohort of 92 MDS patients that the presence of *TET2* and *DNMT3A* mutations confers better response to both azacitidine and decitabine [28].

Whole genome sequencing was instrumental in the identification of a somatic mutation in a DNA methyltransferase gene called *DNMT3A* located on chromosome 2 p23.3. *DNMT3A* catalyzes the addition of a methyl group to the cytosine of the CpG dinucleotides. The amino terminus of the human *DNMT3A* gene contains various motifs that recruit transcriptional repressors. The role of *DNTM3A* is extremely important since this enzyme acts as a check-point of DNA replication by keeping *de novo* methylation mechanisms partially methylated. The methylation of the CpG islands induces repression of downstream genes. The first genetic alteration identified in *DNMT3A* was a frameshift mutation found in a patient with *de novo* AML [29]. *DNMT3A* has been found to be mutated in 8% of MDS cases [30,31]. The interplay between *TET2* and *DNMT3A* has captured the attention of many groups, especially for their role in altering the cytosine methylation and for the fact that current treatments for MDS heavily rely on hypomethylating therapy. *In vitro* murine embryonic stem cells deficient for *dnmt3a* have a competitive advantage compared to cells expressing normal *DNMT3A*, indicating that *DNMT3A* mutations might be clonally dominant [32]. The role of *DNMT3A* in somatic cells is still unknown. Data from mouse models has also suggested a role for *DNMT3A* in epigenetic silencing. Hematopoietic stem cells deficient for *DNMT3A* show differential methylation and up-regulation of multipotency genes. The clinical impact of *DNMT3A* has been correlated with worse OS [33]. Moreover, MDS is usually associated with a hypermethylation and DNMT-inhibitors are associated with increased survival in MDS.

In 2008, parallel massive sequencing identified mutations in the isocitrate dehydrogenase 1 (*IDH1*) gene in gliomas. *IDH1* mutations alter the catalytic activity of the enzyme producing a toxic oncometabolite called *R*-2-hydroxyglutarade which affecting the redox state of the cells, blocking the production of NAPDH. The modality of how *IDH1* promotes the transformation of the normal cells is still unclear. Several studies have postulated that mutations (either by loss of the normal allele or as a result of a dominant-negative effect) might alter the mitochondrial function and lead the cells to use glycolysis. On the other hand, mutations lead to a hypermethylation phenotype with a change in the oxidative metabolism. Mutations in two isoforms of this metabolic enzyme (*IDH1* and *IDH2*) have been found in many solid and blood cancers, including in MDS at a low frequency. This finding suggests that malignancies can be caused by dysfunction in cellular metabolism. A small proportion of late stage MDS cases (5-10%) have *IDH1/2* mutations. In addition, a slightly higher frequency of mutations in this gene is found in sAML patients that evolved from a prior MDS (10-20%). The clinical impact of these mutations is still controversial and varies in different cancers [34]. The higher frequency in patients with higher risk MDS suggests a predisposition to develop AML. (Table 2) It has recently reported that serum measurement of *R*-2-hydroxyglutarade can predict clinical outcome in *IDH1/2* mutant patients [35]. Several studies have indicated an increased risk for relapse in AML patients carrying *IDH1/2* mutations while others have not found any statistical correlation. IDH1/2 proteins are interconnected with TET2 since TET2 seem to be a target of *R*-2-hydroxyglutarate [36]. In addition, *TET2* and *IDH1/2* mutations are mutually exclusive.

V. HISTONE MODIFICATION: ASXL1, RUNX1

ASXL1 (*Additional Sex Combs Like 1*) is a gene located in chromosome 20q11.21. This mutation appears to be independent of the deletion of 20q found in patients with MDS, as *ASXL1* mutations were found in 28% of patients carrying isolated del(20q). *ASXL1* is a member of the Polycomb family which includes genes implicated in chromatin remodeling and gene repression. Indeed, several structural similarities have been identified in genes of the Drosophilidae where *ASXL1* interacts with chromosomal elements. The function of the ASXL1protein is required for the activation and repression of homeotic loci. The protein is believed to disrupt chromatin in localized areas of certain genes while repressing the transcription of other genes. Indeed, mutations lead to the disruption of activating domains (PDH, RARalpha, and SRC1). Monoallelic mutations in *ASXL1* have been found in 16% of high-risk MDS patients and in ~30% of patients with AML and a prior MDS diagnosis. *ASXL1* mutations have been associated with a worse OS in MDS [37]. (Table 2) Mutational analysis has also revealed the co-occurrence of *ASXL* mutations with *TET2*, *IDH1/2*, and *EZH2* mutations, indicating that the epigenetic mechanisms in total are highly dominant in

MDS pathophysiology [38]. The effect of *ASXL1* mutations is still unclear with some studies pointing towards a gain of function and dominant negative effect rather than loss of function.

RUNX1 (*Runt related transcription factor*), also known as *AML1*, maps to chromosome 21q22 and belongs to a family of DNA core binding factors. *RUNX1* regulates the transcription of genes important in hematopoietic stem cell formation during embryogenesis. Indeed, *runx1* knock-out mice die few days after birth without achieving complete hematopoiesis. *RUNX1* translocations produce chimeric proteins, and *RUNX1* is usually highly mutated in AML (M0, M2, and M1 types), while *RUNX1* mutations are found in 20% of MDS cases, mainly t-MDS and radiation exposure [39]. *RUNX1* mutations occur near DNA-binding domains altering the

TABLE II. EFFECTS OF MOLECULAR MUTATIONS ON OVERALL SURVIVAL AND AML TRANSFORMATION IN MYELODYSPLASTIC SYNDROMES AND OTHER MYELOID MALIGNANCIES

Gene	Studies	Disease Group	Overall Survival	AML Transformation
TET2	Tefferi A et al. Leukemia. 2009;23(7):1343-5.	CMML (N=15), MDS (N=7), MDS/MPN (N=3), Others (N=16)	-	-
	Jankowska A et al. Blood. 2009 18;113(25):6403-10	MDS (N=14), MDS/MPN with CMML (N=30), Others (N=24)	no difference	-
	Langemeijer SM et al. Nat Genet. 2009;41(7):838-42	MDS (N=102)	no difference	no difference
	Kao HW et al. 52nd ASH Meeting Abstract 4019	MDS (N=161)	no difference	increased risk
	Jankowska A et al. Blood. 2011 6;118(14):3932-41	CMML (N=52), CMML to AML (N=20)	no difference	-
	Bejar R et al. N Engl J Med. 2011. 30;364(26):2496-506	MDS=439	no difference	-
	Itzykson R et al. Leukemia. 2011;25(7):1147-52	MDS and AML with 20-30% blast (N=86); Tx with 5-aza	no difference	-
DNMT3A	Walter MJ et al. Leukemia. 2011;25(7):1153-8	MDS (N=150)	worse	more rapid progression
	Jankowska A et al. Blood. 2011 6;118(14):3932-41	CMML (N=52), CMML to AML (N=20)	no difference	-
	Lin J et al. PloSOne. 2011;6(10):e26906. Epub 2011 Oct 31.	AML (N=182), MDS (N=51)	worse in MDS	-
IDH1/2	Jankowska A et al. Blood. 2011 6;118(14):3932-41	CMML (N=52), CMML to AML (N=20)	no difference	-
	Bejar R et al. N Engl J Med. 2011. 30;364(26):2496-506	MDS=439	no difference	-
ASXL 1	Gelsi-Boyer V et al. Br J Haematol. 2010;151(4):365-75	CMML (N=53)	worse	increased risk
	Kulasekararaj A et al. 52nd ASH Meeting Abstract 125	MDS (N=45); AML from MDS (N=9), t-AML/MDS (N=7), CMML (N=2)	better if analyzed with *EZH2*	-
	Jankowska A et al. Blood. 2011 6;118(14):3932-41	CMML (N=52), CMML to AML (N=20)	no difference	-
	Thol F et al. J Clin Oncol. 2011 20;29(18):2499-506	MDS (N=193)	worse	decreased time to AML progression
	Bejar R et al. N Engl J Med. 2011. 30;364(26):2496-506	MDS=439	worse	-

Abbreviations. MDS, Myelodysplastic syndromes; MDS/ MPN, Myelodysplastic syndromes/ Myeloproliferative neoplasms; AML, Acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; RARS, refractory anemia with ring sideroblasts; RARS-T, refractory anemia with ring sideroblasts associated with thrombocytosis; LFS, leukemia free survival; - Indicates not available leukemia; Tx, treatment.

TABLE II. EFFECTS OF MOLECULAR MUTATIONS ON OVERALL SURVIVAL AND AML TRANSFORMATION IN MYELODYSPLASTIC SYNDROMES AND OTHER MYELOID MALIGNANCIES (CONTINUED)

Gene	Studies	Disease Group	Overall Survival	AML Transformation
SF3B 1	Visconte V et al. Leukemia. 2012 Mar26(3) :542-5 Epub 2011 Sep 2	MDS MDS/ MPN (N=56)	no difference	-
	Papaemmanuil E et al. NEJM. 2011 Oct 13;365(15):1384-95. Epub 2011 Sep 26	MDS (N=354) MDS (N=354)	better	lower risk
	Malcovati L et al. Blood. 2011 Dec 8;118(24):6239-46	MDS (N=583) MDS/MPN (N=83)	better	lower risk
	Damm F et al. 2012 May;26(5):1137-40	MDS (N=317) clinical data available from 253 patients	no difference	no difference
	Patnaik MM et al. Blood 2012 Jan 12;119(2):569-72 and Am J Hematol. 2013 Mar;88(3):201-6	MDS-RS (N=107)	univariate analysis better but not an independent factor	-
	Visconte V et al. Blood 2012 Oct 18;120(16):3173-86	MDS and MDS/MPN (N=456)	better for MDS and MDS/MPN and within the subset of RARS and RARS-T	-
U2AF1	Yoshida et al. Nature 2011;478: 64-69	MDS MDS/MPN AML (N=582)	-	-
	Makishima et al. 2012;119: 3203-3210	MDS MDS/MPN AML (N=350)	- worse	-
	Graubert et al. Nat Genet. 2012;44: 53-57.	de novo MDS MDS/sAML	no difference	Increased risk
SRSF2	Yoshida et al. Nature 2011;478: 64-69	MDS, MDS/MPN, AML (N=582)	-	-
	Patnaik MM et al. Am J Hematol. 2013 Mar;88(3):201-6	CMML (N=226)	no difference	no difference in LFS
	Kar SA et al. Haematologica. 2013 Jan;98(1):107-13	CMML (N=87)	-	-
	Wu SH et al. Blood. 2012 Oct 11;120(15):3106-11	MDS (N=230)	worse	no impact of the disease evolution
SETBP1	Makishima et al Nat Genet. 2013 Jul 7. In press	MDS, MDS/ MPN, MPN, AML (N=727)	worse	association with myeloid transformation
	Thol F et al. Leukemia. 2013 May 7. In press	MDS and AML (N=944)	no difference	-

Abbreviations. MDS, Myelodysplastic syndromes; MDS/ MPN, Myelodysplastic syndromes/ Myeloproliferative neoplasms; AML, Acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; RARS, refractory anemia with ring sideroblasts; RARS-T, refractory anemia with ring sideroblasts associated with thrombocytosis; LFS, leukemia free survival; - Indicates not available leukemia; Tx, treatment.

DNA core-binding capacity and appear to be independent prognostic factors of worse OS. The mutations induce loss of trans-activation. An elegant study conducted in mice reported that mice transplanted with human cells carrying a mutant *RUNX1* allele develop dysplasia in the erythroid lineage and MDS features.

VI. RNA SPLICING MACHINERY: SF3B1, U2AF1, SRSF2

RNA splicing is an evolutionary mechanism important in maintaining genomic variability. Splicing mutations lead to hereditary diseases, such as X-linked disorders of copper metabolism and retinitis pigmentosa [40]. Somatic mutations in components of the spliceosome machinery were discovered by several groups using whole

genome/ exome sequencing in MDS with ring sideroblasts (RS) and MDS/myeloproliferative overlap syndromes (MDS/ MPN) and later in lymphoid malignancies like chronic lymphocytic leukemia (CLL). *SF3B1* mutations range in frequency, occurring in 68-75% of cases of refractory anemia with ring sideroblasts (RARS) and 81% in RARS associated with thrombocytosis (RARS-T) [41-43] while being rare in other diseases [44]. *SF3B1* has been linked to the pathogenesis of RARS and RARS-T. We associated *SF3B1* mutations to the presence of RS [45,46]. In terms of clinical significance, *SF3B1* mutations have been associated with better OS (P=.01), leukemia-free survival (P=.05), event free survival (P=.008) and with a lower risk of progression to AML [42]. Better OS was noted in RARS/ RARS-T carrying *SF3B1* mutations [45] (Table 2). No difference in OS and AML transformation was found in a cohort of 317 patients with MDS. Patnaik et al. determined that the prognostic value of *SF3B1* mutations was completely accounted for the WHO morphologic grouping [47].

Pharmacologic inhibitors of the spliceosome machinery are currently available [48,49]. We described functional data on Meayamycin, which specifically targets the splicing factor 3b (SF3b) complex [50]. Other pharmacologic compounds including spliceostatin, FR901464, E7107, pladienolide and Sudemycins are currently being investigated. Interestingly, the clinical significance of *SF3B1* in MDS is different from that observed in CLL. The other disease where *SF3B1* is highly mutated is CLL (15%) [51]. It has been reported that *SF3B1* mutations predict worse outcome in CLL patients, and they are commonly associated with deletion of long arm of the chromosome 11, an abnormality present in 5-10% of CLL patients. These findings suggest that different targets might be involved and also that the clonal nature of the two diseases is regulated by different drivers. Our laboratory is actively working in investigating the molecular mechanisms responsible for the good outcomes of patients carrying *SF3B1* mutations focusing attention on DNA damage differences, iron accumulation, mitochondrial targets, and *in-vitro* response to hypomethylating agents. Our preliminary data suggests that the presence of *SF3B1* mutations confer better response rate to erythropoiesis stimulating agents and hypomethylating agents [52].

Mammalian U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) is a heterodimer composed of a 65-kDa subunit ($U2AF^{65}$) and a 35-kDa subunit ($U2AF^{35}$). $U2AF^{65}$ contacts the pyrimidine tract while $U2AF^{35}$ interacts with the AG splice acceptor dinucleotide of the target intron at the 3′ splice site. $U2AF^{35}$ is also known as U2AF1. Recurrent somatic mutations (S34 and Q157) in *U2AF1* were found in 8.7% of patients with primary MDS and were associated with progression to sAML [53,54]. *U2AF1* mutations result in a gain of function. Indeed, a significant increase in exon skipping was observed when the mutant p.Ser34Phe cDNA was transiently expressed *in vitro* [53]. Pattern of splicing for *U2AF1* has recently been elucidated [55].

SRSF2 encodes a member of the serine/arginine (SR)-rich family of pre-mRNA splicing factors with an RNA recognition motif (RRM) and a RS domain. *SRSF2* is believed to be a key element in the acetylation/ phosphorylation network and an important regulator of the DNA stability. The first report describing *SRSF2* mutations in CMML found mutational frequencies ranging between 28.4-47% [56]. The most common alteration occurs at amino acid position Prolin95 between the RRM motif and the RS domain. *SRSF2* has been indicated as a new diagnostic target in CMML. The presence of *SRSF2* mutations correlate with higher age, anemia, and normal karyotyping and do not seem to impact survival. Other studies associated *SRSF2* mutations with poor outcomes (Table 2). The impact of baseline spliceosomal mutations were investigated in a cohort of patients that underwent allogeneic hematopoietic cell transplantation, finding that *SRSF2* mutants had similar outcomes to wild type patients, suggesting that transplant can compensate for the adverse impact of this gene [57].

VII. INFREQUENT MOLECULAR MUTATIONS

We have discussed the genes for which the clinical impact has been intensively studied and correlated with the patient phenotypes. Several of the above genes are relevant because they can serve as therapeutic targets in MDS, like hypomethylating agents and histone deacetylase. On the other hand, there are numerous other genes with lower mutational frequencies reported in MDS and other related malignancies in the same biological pathways discussed above. Mutations in other genes regulating histone function and members of the polycomb group are less frequent in MDS. *EZH2* acts as histone methyltransferase that normally works in maintaining the repressed status of TSGs and has been found mutated in 2-6% of patients with MDS [58]. *UTX*, a demethylase gene located in the X chromosome, has been actively interrogated for mutations but they have only been found in MDS/ MPN patients at a frequency of 6%. Mutations in *c-CBL*, *CBL-b*, and *CBL-c* are extremely rare in MDS (1%), while present in a much higher frequency in patients with MDS/ MPN (8.1%) [59]. Infrequent mutations in other spliceosomal genes, such as *U2AF65*, *ZRSR2*, *SF1*, *SRSF1*, *SF3A1*, *PRF40B*, *PRPF8*, and *LUC7L2*, were also detected [54]. Mutations in *N/KRAS*,encoding proteins homologous to RAS, were reported at frequencies of 2-6%. Several other genes have been found but are even less commonly dysregulated and/ or mutated in MDS like *IER3*, a sentinel of cellular stress, *FLT3*, a class III receptor tyrosine kinase which determines proliferative advantage if mutated in AML, and *CDC25c*, a gene in the cell division pathway that interacts with MAP kinase family members for which the frequency of mutations is unavailable.

VII. BRIEF DESCRIPTION OF GENES ASSOCIATED WITH SPECIFIC CHROMOSOMAL ABNORMALITIES: TP53 AND RPS14

Gene mutations can be associated with specific classes of MDS patients carrying distinct chromosomal anomalies. Mutations in *TP53*, a TSG, have been correlated with anomalies of chromosome 5 (isolated del5q, -5/5q-), 17p-, and complex karyotypes, suggesting different biological and driving mechanisms. Mutations were detected early in the MDS presentation, suggesting a primary event in the manifestation of the disease. Associations of *TP53* mutations and t-MDS have been reported. Moreover, mutations have also been correlated with response to therapy (5-azacytidine) and seemed to disappear upon response [60].

The discovery of the haploinsufficiency of *RPS14* and its somatic deletion in patients with 5q-syndrome associated MDS to other ribosomopathies and correlated this ribosomal protein to the pathogenesis of 5q- patients. In addition, *RPS14* seems to be a major player in the anemia of patients with 5q- and a marker of good prognosis [61].

IX. NEWLY DISCOVERED MOLECULAR MUTATIONS

Whole exome sequencing aided in the discovery of mutations in *SETBP1* with a higher frequency in atypical CML (aCML). Germline alterations in *SETBP1* are characteristic of the Schinzel-Giedion midface retraction syndrome. Heterozygous variants, commonly found in a germline configuration, are found to be somatic in aCML [62]. Direct sequencing of *SETBP1* in different subsets of MDS, MDS/ MPN, and other diseases found a lower frequency in MDS (2.2%) [63]. *SETBP1* mutations have been correlated with leukemogenesis [64] and found to be associated with the presence of other two genes which correlate with a negative prognosis (*ASXL1* and *EZH2*) and with two deleterious chromosomal abnormalities (iso17(q) and -7).

X. CONCLUSION

High-resolution genomic technologies have discovered a myriad of somatic alterations in different genes with a variety of functions in MDS and other hematologic malignancies. Even in the era of genomic technologies, MC still represents the gold standard in the identification of chromosomal abnormalities in MDS and still holds a fundamental role in the prognostic scoring systems. Molecular mutations represent a new parameter to take into account in the risk stratifications models. Whole genome/ exome sequencing represents a platform of discovery for new markers of MDS, but the role and the mechanisms explaining the heterogeneity of the clinical/ morphologic manifestations of MDS is still unknown. On the other hand, even with the advent of all these sophisticated technologies conventional Sanger sequencing still represents the most reliable and cost affordable technique. Pitfalls including artefacts/ false negatives are also commonly faced using the next generation technologies as mentioned above. Lower-frequency molecular mutations are also important in MDS, but harder to study in terms of prognostic and predictive significance. Nonetheless, they may provide important insight in disease biology unique to the specific individual carrying the rare mutation. Recurrence of somatic alterations is a pre-requisite to define a specific gene as a possible candidate in the pathogenesis of a disease subtype. We have interrogated hundreds of possible candidates selected after performing whole exome sequencing in MDS and other related disorders, finding sporadic somatic alterations with no detectable frequency in large cohort of same disease type. Germ-line sources (lymphocytes and/ or buccal swabs when possible), scoring of the genetic variants based on an acceptable coverage, stringent bioinformatics algorithms, searching in public database for SNPs exclusion, absence of variants in healthy individuals, and further confirmation of the eventual alterations with bidirectional sequencing are all useful tasks to generate valid results.

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